Journal of Food Protection, Vol. 63, No. 7, 2000, Pages 867–870

Commercial Field Trial Evaluation of Mucosal Starter Culture To Reduce *Salmonella* Incidence in Processed Broiler Carcasses

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MS 99-378: Received 22 December 1999/Accepted 17 February 2000

ABSTRACT

A series of four paired-house studies was conducted in Arkansas, Alabama, and Georgia (two farms) to determine the efficacy of Mucosal Starter Culture (MSC) in eliminating or reducing salmonellae in broiler chickens. Randomly designated chicks were treated twice with MSC. First they were sprayed with an MSC solution using a spray vaccination cabinet in the hatchery, and then they received MSC in the first drinking water at the growing house. Chicks were grown in identically constructed and equipped paired houses managed by the same grower. At the end of grow-out, broilers were tested for the presence of salmonellae on the farm and during processing. In three trials where no hatchery salmonellae were found, less salmonellae were found on MSC-treated chickens compared to untreated chickens. On the farm at the end of grow-out, salmonellae were detected in 54 of 150 untreated control chickens compared to 40 of 180 MSC-treated chickens. In the processing plant, significantly ($P \leq 0.05$) more salmonellae were detected on prechill untreated control carcasses (23 of 180) compared to MSC-treated carcasses (12 of 180) and on untreated postchill processed carcasses (9 of 180) compared to MSC-treated carcasses (0 of 180). In one trial where appreciable (28% of egg shell samples) salmonellae was found before treatment with the MSC, more salmonellae were found in the treated birds than in the control birds both on the farm and after processing. These data confirm that when salmonellae levels were controlled in the hatchery, a significant reduction in the salmonellae was found on processed broiler carcasses treated with MSC and that this reduction in salmonellae was carried through processing to the final processed carcass, thus potentially reducing consumer exposure to salmonellae.

Control of salmonellae in an integrated poultry operation is complex because there are numerous potential sources of salmonellae contamination including chicks, feed, rodents, wild birds, insects, transportation, and the processing plant environment. Effective control of salmonellae will require that intervention strategies be adopted for each of these sources. For posthatchery salmonellae sources, competitive exclusion treatment of day-of-hatch chicks, as first described by Nurmi and Rantala *(11)* and reviewed by Bailey *(1)*, has been used to help control intestinal colonization of broilers by salmonellae. Several researchers including Mead and Impey *(10)* in England, Goren and coworkers *(9)* in the Netherlands and Stavric *(13)* in Canada expanded the knowledge of effectiveness and practical applications of competitive exclusion treatments.

Bailey and coworkers *(2)* showed that most commercially used anticoccidial and antimicrobial feed additives did not adversely affect the efficacy of an undefined competitive exclusion product developed in their laboratory. Stern *(14)* described the process for making a competitive exclusion product derived from the mucosal scrapings of pathogen-free adult broiler chickens (mucosal competitive exclusion, MCE). Large-scale commercial field trials were conducted in Puerto Rico and Georgia to test the efficacy of the MCE to protect broiler chickens against natural salmonellae colonization of the intestinal tract and subsequent contamination of the processed chickens from these treated flocks. In Puerto Rico, incidence rates of intestinal colonization with salmonellae were reduced from 11% in controls to 2% in MCE-treated flocks and from 41% in controls to 10% in treated flocks on processed carcasses *(6)*. In Georgia, incidence rates of intestinal colonization were reduced from 2% in controls to undetectable in treated and from 9.5% of control to 4.5% of treated processed carcasses *(5)*. The process for making MCE was issued a U.S. Patent (no. 5,451,400) in 1995 and was licensed to the Continental Grain Company in 1996 to develop a commercial product. This product is now called Mucosal Starter Culture (MSC). The current study was initiated to determine the efficacy of MSC to reduce salmonellae on processed broiler carcasses in normal commercial operations.

MATERIALS AND METHODS

Treatment groups. Each trial consisted of paired growing houses that were of the same design, had the same types of equipment, used feed from the same lots, and in which identical management practices were used. Houses in each pair were randomly designated as control or treated. Approximately 2 to 3 weeks prior to initiation of each trial, 5 to 16 paired houses in each trial location were sampled by drag swabbing to identify those houses that had salmonellae present in the previous flock, and only those houses were used in these trials. Both houses were treated as if they received MSC culture in the first water, thereby not disclosing the treatment group to the grower. Samples were collected by the research laboratory personnel.

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Grow-out. Depending on location, each house was filled with from 17,000 to 23,000 1-day-old straight run (approximately 50% male and 50% female) Hubbard, Arbor Acres, Avian Farms, Peterson, Cobb, and/or Ross chicks with an initial body weight of 35 to 45 g. One randomly selected house contained MSC-treated chicks and the other contained control saline-treated chicks. Chicks from two breeder houses were randomly divided after hatch and prior to administration of MSC into treatment and control groups with equal numbers of chicks for each group from each breeder farm. All chicks were given Marek's vaccine in the hatchery. Starter and grower feed contained anticoccidial drugs as indicated in the feed section. No other vaccine or medications were needed in these studies.

Study facilities. Studies were conducted on commercial broiler production farms in Arkansas, Alabama, and Georgia. All trials were conducted in typical broiler houses (12.2 by 122 m) with incandescent lighting, gas brooders, and one space heater per house. Houses were cooled with eight positive-pressure 91-cm fans. Feed was provided through two lines of Chore-Time pan-type automatic feeders and water with Agri-dry cup drinkers. Space was allocated to the industry standard of about 0.065 m^2 per bird.

Feed formulations. Chickens were given starter crumbles, finisher pellets, and withdrawal pellets feed ad libitum. Feedstuffs included: corn, soybean meal, animal protein, poultry protein, animal/poultry fat, lysine, methionine, phosphate, calcium carbonate, betaine, choline chloride, and salt. Feeds were supplemented with proprietary mixtures of vitamin and mineral premixes. Feed additives included one or more of the following salinomycin, roxarsone, bacitracin-md, copper sulfate, ethoxyquin, virginiamycin, propionic acid, and bambermycins. All feeds used in this study met typical industrial nutrient levels. Water was provided ad libitum via cup drinkers.

MSC administration. MSC was administered twice on the day of hatch. First, a vaccine applicator spray cabinet was used to spray chicks with about 0.2 ml of 10^7 MSC organisms per ml solution after placement in the transport container. Then, each chick was supplied with an average of 10 ml of an MSC water solution containing 10^6 MSC organisms per ml as the first drinking water.

General management practices. Birds were placed on built-up (used) litter in growing houses covered with a 5-cm top-dressing of clean shavings. Growers worked houses at least twice each day. On each visit, temperature was monitored, waterers and feeders were checked, and dead birds were removed and recorded. Air flow was controlled by tunnel fans and birds were cooled by evaporative coolers or heated by gas heaters. Feed was supplied to chicks automatically via auger from feed storage bins at each house. Feed and water were available ad libitum. No birds were removed from any part of the study unless they died. A normal early chick mortality of 1 to 3% was observed in these studies. Dead birds were composted or incinerated.

Variable measured. The incidence of salmonellae at the end of grow-out and after processing was the primary variable of interest. At the end of grow-out, presence of salmonellae was measured by aseptically sampling ceca from 60 randomly selected chickens per house 1 to 2 days before birds were transported to the processing plant. In the processing plant, 60 carcasses per house were sampled for salmonellae before and after chilling by the whole carcass rinse technique *(8)*. A comparison of the percentage of salmonellae-positive ceca from the grow houses and the rinse samples in the processing plant was made between the birds from the treated and untreated (control) houses. In order to minimize effects of processing plant cross-contamination, the treated house were processed as the first flock of the day on one day and the control house was the first flock processed the next day.

Salmonellae detection methods. One to 2 days before the end of grow-out, both ceca from 60 randomly selected chicken from each house were aseptically removed from birds that had been killed by cervical dislocation. Ceca were placed into stomacher bags and shipped overnight to the Russell Research Center. In the processing plant, 60 randomly selected broiler carcasses were pulled from the line before and after the immersion chiller. Each carcass was placed in a sterile bag and rinse sampled with 100 ml sterile water *(8)*. The rinse solution was poured into sterile specimen containers and shipped to Russell Research Center. On the day of receipt in the laboratory, ceca were separated and a volume of universal preenrichment broth (Difco, Detroit, Mich.) *(4)* equal to three times the weight of the ceca added to each stomacher bag. Concentrated universal preenrichment (10×) was added to the carcass rinse fluid to make a single strength medium. Universal preenrichment tubes were incubated for 24 ± 2 h at 35°C and then 0.1 ml of the universal preenrichment solution was transferred to 10 ml TT broth. After incubation for 24 ± 2 h at 42 C, the VIDAS (bioMerieux/Vitek, St. Louis, Mo.) automated immunoassay was used according to the instructions of the manufacturer to screen each sample for salmonellae. Samples that gave a positive screen on the VIDAS assay were streaked from the TT broth onto BG sulfa and modified lysine iron agar plates for isolation of salmonellae. Typical CFUs were serologically confirmed to be salmonellae by a latex agglutination assay. Data were recorded as positive or negative for the presence of salmonellae in each sample.

Data analysis. The presence or absence of salmonellae was measured on the farm by analysis of ceca from birds 1 day prior to processing and at the processing plant by analysis of whole carcass rinses before and after chilling. The odds ratio was calculated for each of the three test farm and associated processing plant samples from farms not showing high levels of salmonellae in the hatchery samples. The odds ratio was then studied using the Breslow/Day test to determine homogeneity *(12)*. When the odds were found to be heterogenous, data were examined as three separate groups (three farms). When the odds ratios were homogenous, the data were pooled and examined together to calculate a common odds ratio. A confidence interval was then established to report the magnitude of any treatment effect observed. In addition, chi-square analysis was done to test for a significant relationship between treatment (MSC or control) and number of positive samples.

RESULTS AND DISCUSSION

Mortality was less than 2% in all houses, with no differences between MSC-treated and control birds. In the Arkansas, Alabama, and the second Georgia trial, none of the 25 random shell samples taken from the flocks during hatching was salmonellae positive. In the first Georgia trial, 7 of the 25 shell samples (28%) were salmonellae positive. Both on the farm and after processing, MSC-treated broilers from this trial had equal to or more salmonellae than the untreated control broilers. It is recognized that competitive exclusion cultures do not effectively reduce salmonellae colonization in chickens if the salmonellae gets into the

TABLE 1. *Effect of MSC[a] treatment on salmonellae incidence in cecal samples from farms stocked with chicks that were* Salmonella *negative in the hatchery*

Location	Salmonellae (+)/ total tested[b]		
	Control	MSC treated	Chi square
Arkansas	24/60	6/60	$P = 0.01$
Alabama	30/60	34/60	$P = 0.46$
Georgia no. 2	0/60	0/60	n/a

[a] MSC, Mucosal Starter Culture.

[b] Samples were taken on the farm the day before processing (6 weeks of age).

chickens via the hatchery before the competitive exclusion cultures are applied *(3, 6).* These data again demonstrate that as with other competitive exclusion products, when salmonellae contamination is present before the competitive exclusion microflora, significant protection from salmonellae colonization is difficult to achieve.

When the MSC was given to the chicks before they were exposed to salmonellae however, significant protection was provided (Tables 1 and 2). A trend was seen on the farm where broilers carrying salmonellae in their intestinal tract were reduced in controls from 54 of 180 tested to 40 of 180 MSC-treated broilers tested (Table 1). When the results from the three farms (ceca from birds 1 day before processing) were examined with the Breslow-Day test, they were found to be heterogenous with a likelihood ratio of 0.0005. Therefore, these data were not pooled but were examined as three separate groups. When examined individually, one farm (Georgia no. 2) had no salmonellae in either control or treated ceca, one farm (Arkansas) had a significant effect of treatment (by chi square $P = 0.01$), and one farm (Alabama) had essentially no difference (by chi square $P = 0.46$).

Before the chiller, salmonellae were reduced on untreated controls from 23+ of 180 tested to 12+ of 180 in the MSC-treated groups. Data from carcass rinse samples taken prechill were found to be homogeneous by the Breslow-Day test with a likelihood ratio of 0.8267. Therefore, these data were pooled and a common relative risk with confidence intervals was calculated by the Mantel-Haenszel test. The value for the relative risk (treatment/control) was 0.488 with 95% confidence bounds of 0.237 and 1.002. Chi-square analysis of these data showed that there was a significant relationship ($P = 0.05$) between treatment with MSC and resulting reduction in the presence of salmonellae on processed prechill broiler carcasses.

After chilling, salmonellae were reduced on untreated controls from 9+ of 180 tested to 0+ of 180 in the MSC-treated groups. Data from carcass rinse samples taken postchill were found to be homogenous by the Breslow-Day test with a likehood ratio of 0.826. Therefore, these data were pooled and a common relative risk with confidence intervals was calculated by the Mantel-Haenszel test. The value for the relative risk (treatment/control) was 0.950 with 95% confidence bounds of 0.919 and 0.982. Chi-square analysis of these data showed that there was a significant relationship ($P = 0.02$) between treatment with MSC and resulting reduction in presence of salmonellae on processed postchill carcasses.

TABLE 2. *Effect of MSC[a] treatment on salmonellae contamination of pre- and postchill carcasses from Arkansas, Alabama, and Georgia farms with no salmonellae in the hatchery sampled in the processing plant*

Location	Salmonellae (+)/total tested		
	Control	MSC treated	Chi square
Pre-chill	23/180	12/180	$P = 0.05$
Post-chill	9/180	0/180	$P = 0.02$

[a] MSC, Mucosal Starter Culture.

Reduction in the presence of salmonellae on both pre- and postchill carcasses was consistent for all three field trials. In the Alabama field trial, however, no differences were seen in salmonellae between control and MSC-treated carcasses on the farm. The most likely explanation for this is that only salmonellae qualitative data were generated in these trials. It is probable that the levels of salmonellae found in the MSC-treated on farm birds was much lower than that of the control birds and these lower levels of salmonellae resulted in less contaminated processed carcasses. For the last 25 years, competitive exclusion cultures have been demonstrated in the laboratory and in commercial preparations to reduce salmonellae in broiler chickens on the farm *(5, 7, 8).* Blankenship and coworkers *(4)* showed that the seed material for MSC, mucosal competitive exclusion, could reduce salmonellae both in the growing house and on processed carcasses. The present field trials are the first with a commercial preparation, MSC, made from the MCE seed culture. In addition, the previously reported field trial results with MCE *(6)* and this trial with MSC are the only reports in the literature to show that this significant reduction in salmonellae can be carried from the field, through processing, to the final processed carcass, thus reducing consumer exposure to salmonellae.

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